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Photoperiod and temperature effects on root cold acclimation.

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PHOTOPERIOD AND TEMPERATURE EFFECTS ON ROOT COLD ACCLIMATION

A Thesis Presented

By

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Plant and Soil Sciences

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Acknowledgement is also made to the faculty, staff, and all who aided in the completion of my work.

ABSTRACT

PHOTOPERIOD AND TEMPERATURE EFFECTS ON ROOT COLD ACCLIMATION

(August 1976)

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Intact roots of *Potentilla fruticosa* L. cv. Katharine Dykes and *Picea glauca* Voss were studied during the autumn and early winter of 1975 to determine the importance of photoperiod and temperature in cold acclimation. Results indicated that short days and cool temperatures were necessary for maximum acclimation. Sub-zero air and root medium temperatures did not increase the rate of cold acclimation.

Potentilla roots were counted and measured in late autumn to determine the relationship between photoperiod and temperature for root growth. It appears that long days stimulate root initiation, while warm temperatures enhance root elongation.

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SECTION I

PHOTOPERIOD AND TEMPERATURE EFFECTS ON ROOT COLD ACCLIMATION

PHOTOPERIOD AND TEMPERATURE EFFECTS ON ROOT COLD ACCLIMATION[1]

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Additional index words: root cold hardiness, *Potentilla fruticosa*, *Picea glauca*

Abstract. Intact roots of *Potentilla fruticosa* L. cv. Katharine Dykes and *Picea glauca* Voss were studied during the autumn to determine the importance of photoperiod and temp in cold acclimation. Short days and cool temp were necessary for max cold acclimation of roots of these species.

Studies on the cold acclimation of plant roots have been limited to date, but it is generally accepted that shoots of plants withstand a lower temp than roots (1, 5, 6, 12). With increased use of containers for nursery crops, however, root cold hardiness may be more important for winter survival than shoot hardiness.

Temperatures just above freezing are necessary to induce max rates of cold acclimation of roots (5, 13). Minimum air temp and hardiness may be related during the period of greatest cold hardiness (5). Reduced moisture content of roots appears to be related to hardiness (10, 11), although moisture differences between stems and roots do not account for their acclimation (6, 9). Root hardiness does not appear to be affected by soil N (9), plant N (4, 7, 10),

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1 Received for publication

2 Graduate Research Assistant and Professor, respectively.

plant P (7), plant K (4), or changes in sugars (10). Roots farther from the stem and younger are less hardy than those closer to the stem and older (5, 8). Mityga and Lanphear (5) concluded that the youngest roots of *Taxus* did not have the capacity to harden, and this was confirmed in *Pyracantha* (13). We have found no reports of the effect of light on cold acclimation of roots.

The objective of this study was to determine the importance of short days and temp on the cold acclimation of roots of *Picea* and *Potentilla*.

Materials and Methods

Three-year seedlings of *Picea* (a narrow-leaved evergreen tree) and rooted cuttings of *Potentilla* (a deciduous, woody shrub) were transplanted into 15.24 cm pots containing 1 peat:1 sand (v/v). During the mixing process 2.46 kg of 9-month Osmocote (18-1.3-5), 2.95 kg superphosphate, 2.95 kg lime, and 55.9 g fritted trace elements were added per m^3 of medium. The potted plants were placed in a container area under normal seasonal growing conditions on May 7, 1975.

Treatments were outdoor, normal storage, lighted storage, and warm storage. A summary of the treatment conditions is given in Table 1. Air and medium temp at each of the treatment locations were measured by thermistors through a specially constructed switching box, and recorded on Rustrac recorders. Minimum air and media temp for the 4 acclimating conditions are shown in Fig. 1 and 2. The outdoor plants were exposed to freezing 10 times and the medium reached a min temp of $0^{\circ}C$. Plants in normal storage and lighted storage did not freeze and plants in the warm treatment were kept above 15°.

Freezing tests were made on Aug. 1, Sept. 15, Oct. 1, Nov. 1, and Dec. 1 to determine cold acclimation of the roots. Five plants in pots were tested at each of several temp, at 2.8°C intervals for each treatment and date. Five unfrozen plants provided controls. The roots of intact plants were frozen in a circulated methanol-water bath and thawed according to the procedure of Havis (3). After thawing, the *Picea* plants were placed under normal storage conditions until Jan. 20, when the cold requirement had been met, then transferred to a greenhouse for growth. The *Potentilla* plants, which did not have a dormant or rest period, were placed directly into growing temp conditions for evaluation.

Secondary mature roots, as described by Mityga and Lanphear (5), were evaluated by subjective visual analysis. Roots were rated for browning, and also regrowth from the secondary mature roots. Cold hardiness was evaluated as the lowest temp at which at least 50% of the secondary mature roots survived. Results of the root evaluation were supported by initiation and maintenance of new shoot growth.

Results and Discussion

Roots of *Potentilla* plants in the outdoor treatment developed cold hardiness from -3.9°C on Aug. 1 to -6.7° on Sept. 15, and continued acclimating to -15.0° on Dec. 1 (Fig. 3). Roots in normal storage acclimated the same as those out-of-doors which were exposed to freezing air temp. Roots in lighted storage did not change from the Aug. 1 (-3.9°) level of cold hardiness until Nov. 1, when they acclimated to -6.7°. This level of cold hardiness was maintained on the Dec. 1 test date. *Potentilla* roots in the warm treatment did not increase in cold hardiness during the test period.

Picea roots in the outdoor treatment were killed by a temp of -3.9°C on Aug. 1, and did not increase in cold hardiness below -3.9° until after Oct. 1, but finally acclimated to -23.3° by Dec. 1 (Fig. 4). Roots in normal storage acclimated similarly to roots in the outdoor treatment to a max of -20.6° on Dec. 1. Roots of plants in the lighted storage did not increase in cold hardiness below -3.9° until Nov. 1, and then acclimated to a max of -12.2° on Dec. 1. Roots of *Picea* in the warm treatment acclimated to -6.7° by Oct. 1, yet developed no more cold hardiness than roots in the lighted storage treatment on Dec. 1.

On Aug. 1, when both species were in active growth, *Potentilla* roots were slightly hardier than *Picea* roots (Fig. 3 and 4). By Dec. 1, however, *Picea* roots (-23.3°C) were considerably hardier than *Potentilla* roots (-15.0°) under outdoor conditions. Maximum cold hardiness of both species was near -30° in Jan. (data not shown).

These results indicate that cold acclimation of roots of *Potentilla* and *Picea* can be accomplished nearly as well at min air temp of 2°C as at below 0°, with natural autumn day lengths. Under the long day regime in the lighted storage at 2° min temp, some cold acclimation took place but not as much as under natural photoperiod. *Potentilla* continued terminal shoot growth in the lighted storage, whereas the tops of plants in normal storage became fully dormant. The continued terminal shoot growth may have resulted in auxin transport to the roots which stimulated growth, and prevented acclimation. *Picea* plants exhibited sporatic shoot growth under the lighted storage condition while there was no shoot growth under the normal storage condition.

Warm treatment plants of Potentilla were exposed to shortening day lengths, but did not increase in cold hardiness, indicating that cooler temp were necessary for cold hardiness induction. Picea warm treatment plants, however, did acclimate to a level equal to the plants in the lighted storage condition. Apparently, in Picea, cold hardiness was induced by either shortening photoperiod or cold experience, and to attain the greatest cold hardiness, both factors were required. Under warm treatment conditions it is likely that the relatively high temp kept the roots in a growing condition. If cold acclimation is associated with cessation of growth as was indicated for shoots by Fuchigami, et al. (2), the reduced acclimation found in this treatment would be expected.

Mityga and Lanphear (5) indicated a close relationship between min air temp and root hardiness. Our results are in agreement since both outdoor and normal storage treatments resulted in greater root hardiness than warm storage. The lower outdoor air temp (Fig. 1) did not, however, produce greater hardiness than the normal storage temp.

The results of this study suggest that a different cold acclimation response mechanism is involved for each species. Cold acclimation in Potentilla appeared to be induced by cold and reinforced by a shortening photoperiod, while Picea seemed to be induced by either cold or shortening photoperiod with each mechanism reinforcing the other. A possible explanation for the differences in cold acclimation mechanisms may lie in the fact that Picea has a dormant or rest period, while Potentilla does not. If the induction of rest brings about a corresponding increase in hardiness, the shortening day length, which normally induces the rest period, could have increased root hardiness in the warm storage Picea, but not in Potentilla.

If evergreens, in general, can acclimate without cool temp, nurserymen may place these plants into winter storage earlier in the autumn. This would eliminate the problem of early frost injury to plants left outside for acclimation purposes.

Table 1. Cold acclimation treatments.

Treatment	Min air temp (°C)	Photoperiod	Initiation dates	Location
Outdoor	ambient (to -3.9°)	Natural[z]	May 7	Cold frame
Normal storage	2	Natural	Sept. 12	Poly house
Lighted storage	2	14.5 hrs (2100 lux incandescent)	Aug. 3	Poly house
Warm storage	15	Natural	Sept. 4	Glass house

[z] 14.5 to 9.3 hrs

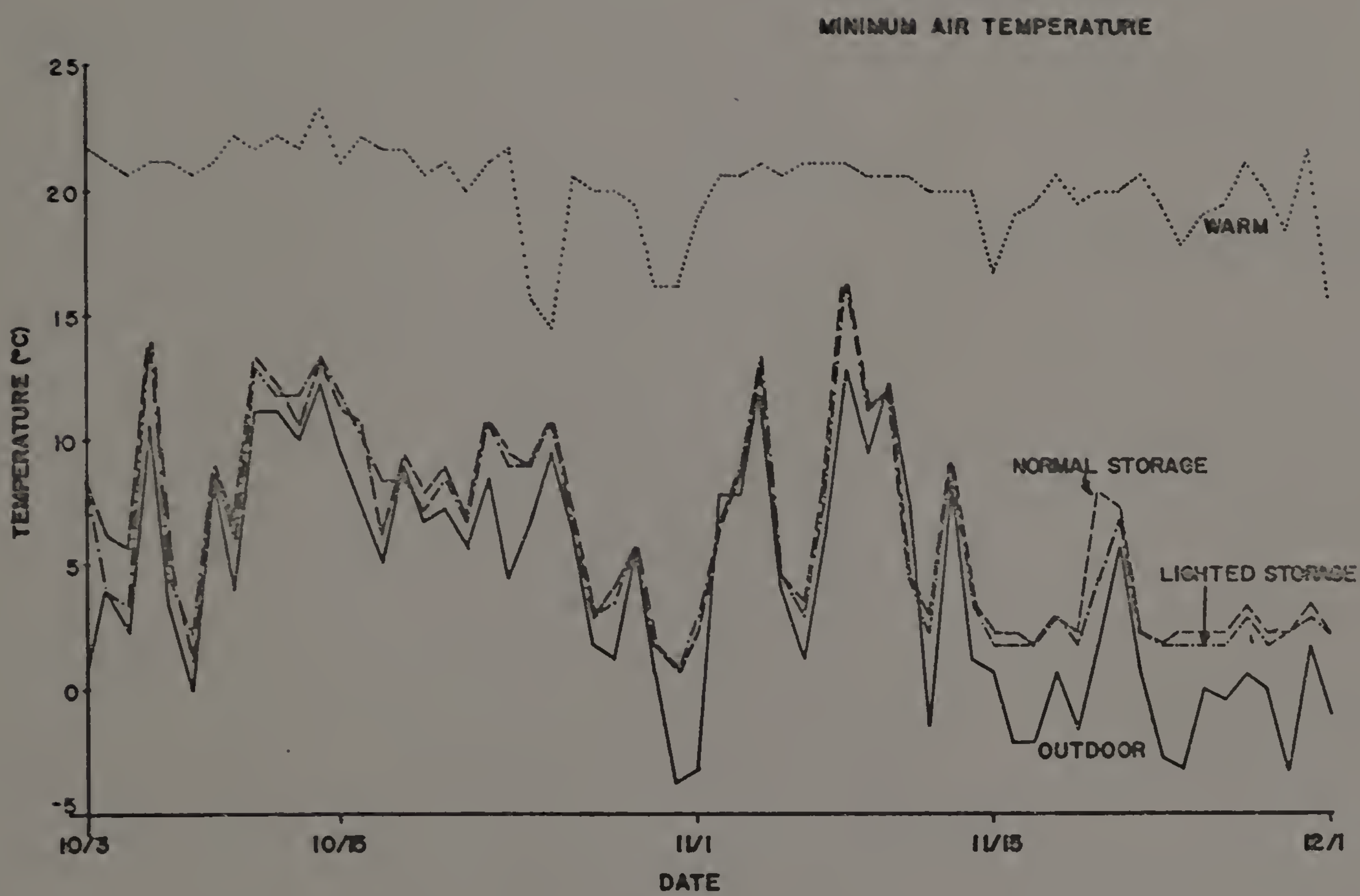

Fig. 1 Minimum daily air temp exposure of Potentilla and Picea plants in storage regimes.

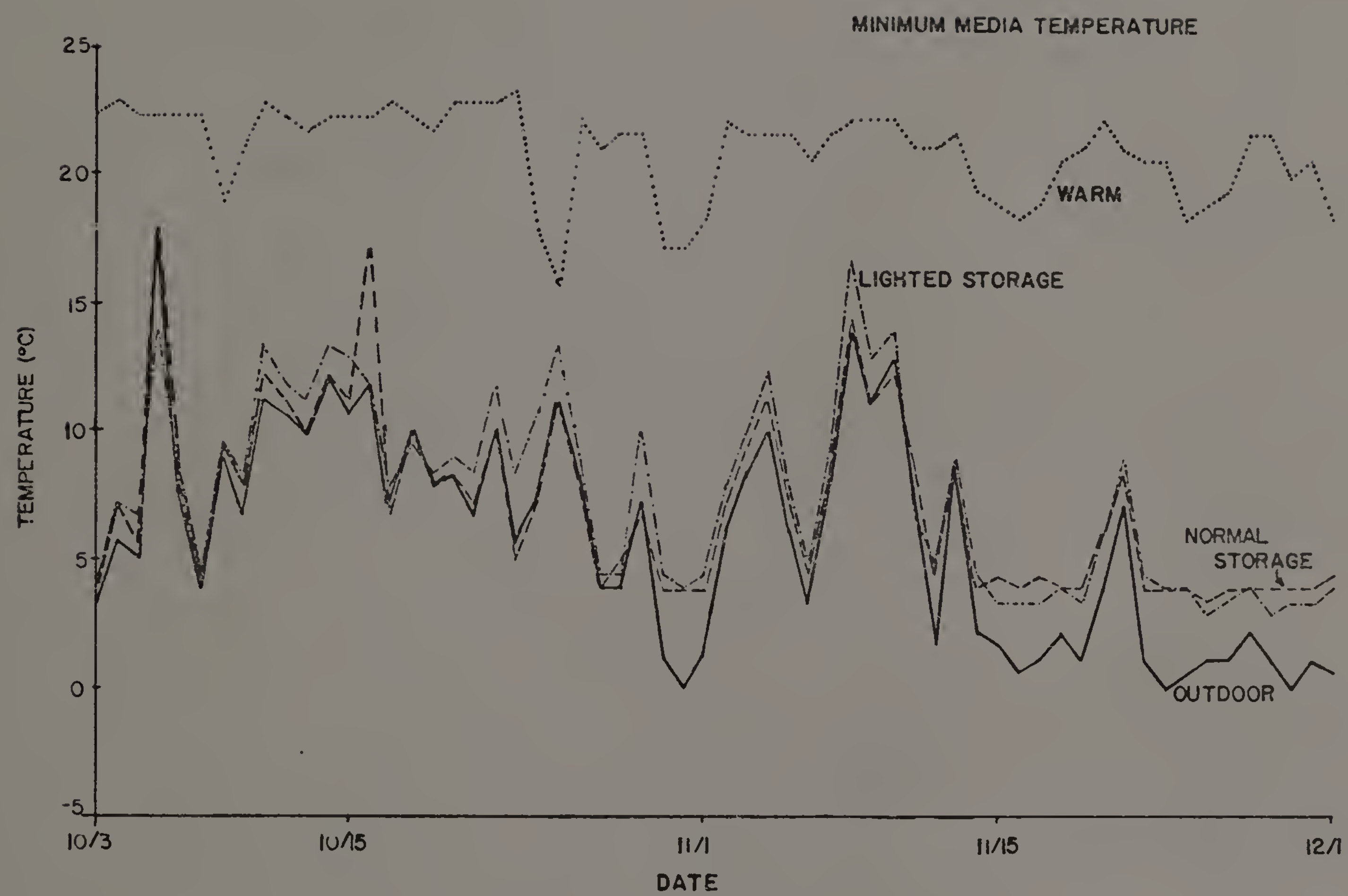

Fig. 2 Minimum daily medium temp exposure of Potentilla and Picea plants in storage regimes.

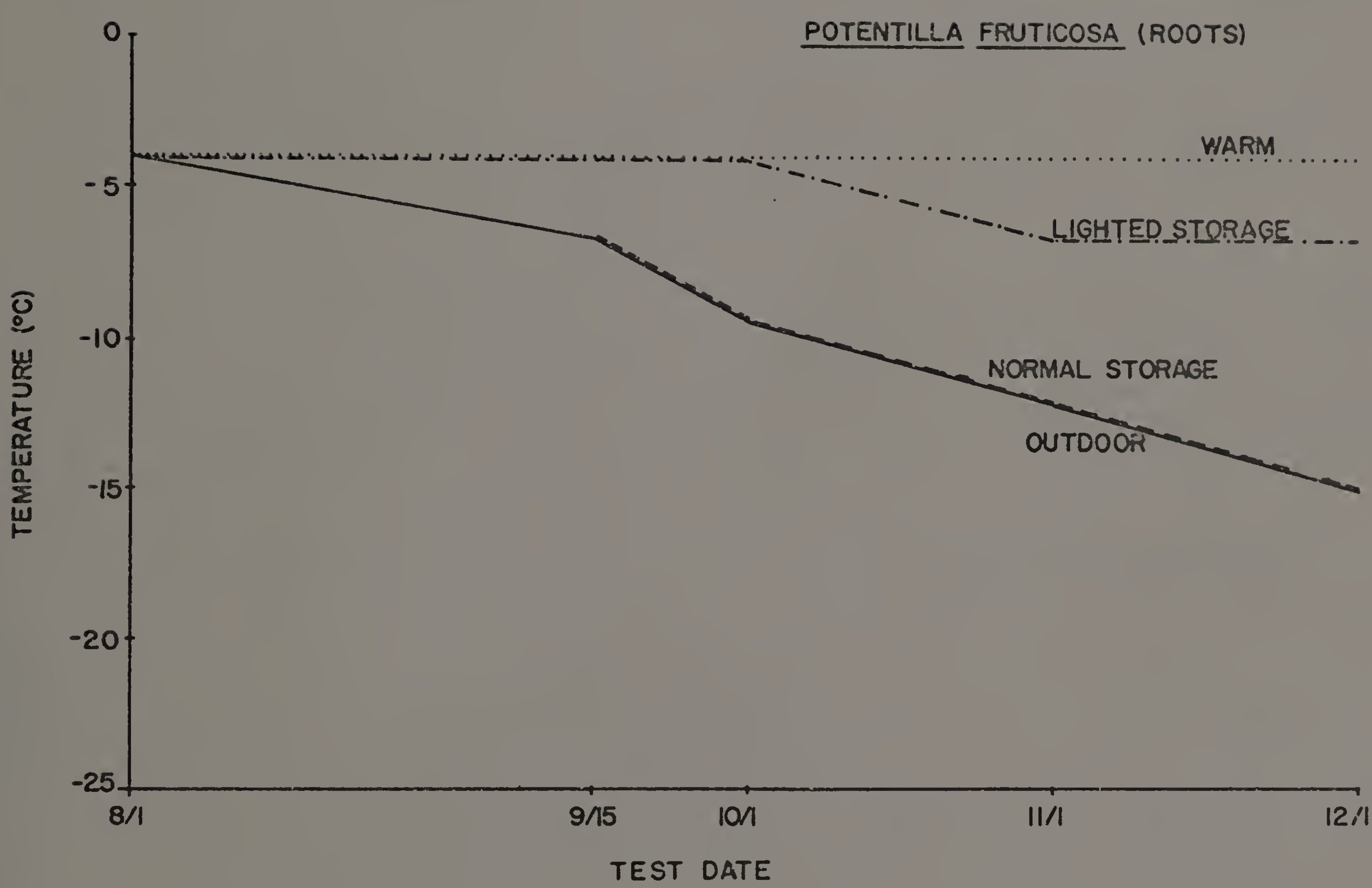

Fig. 3 Cold acclimation of Potentilla roots in the storage regimes described in Table 1. Lowest temp at which 50% or more secondary mature roots survived.

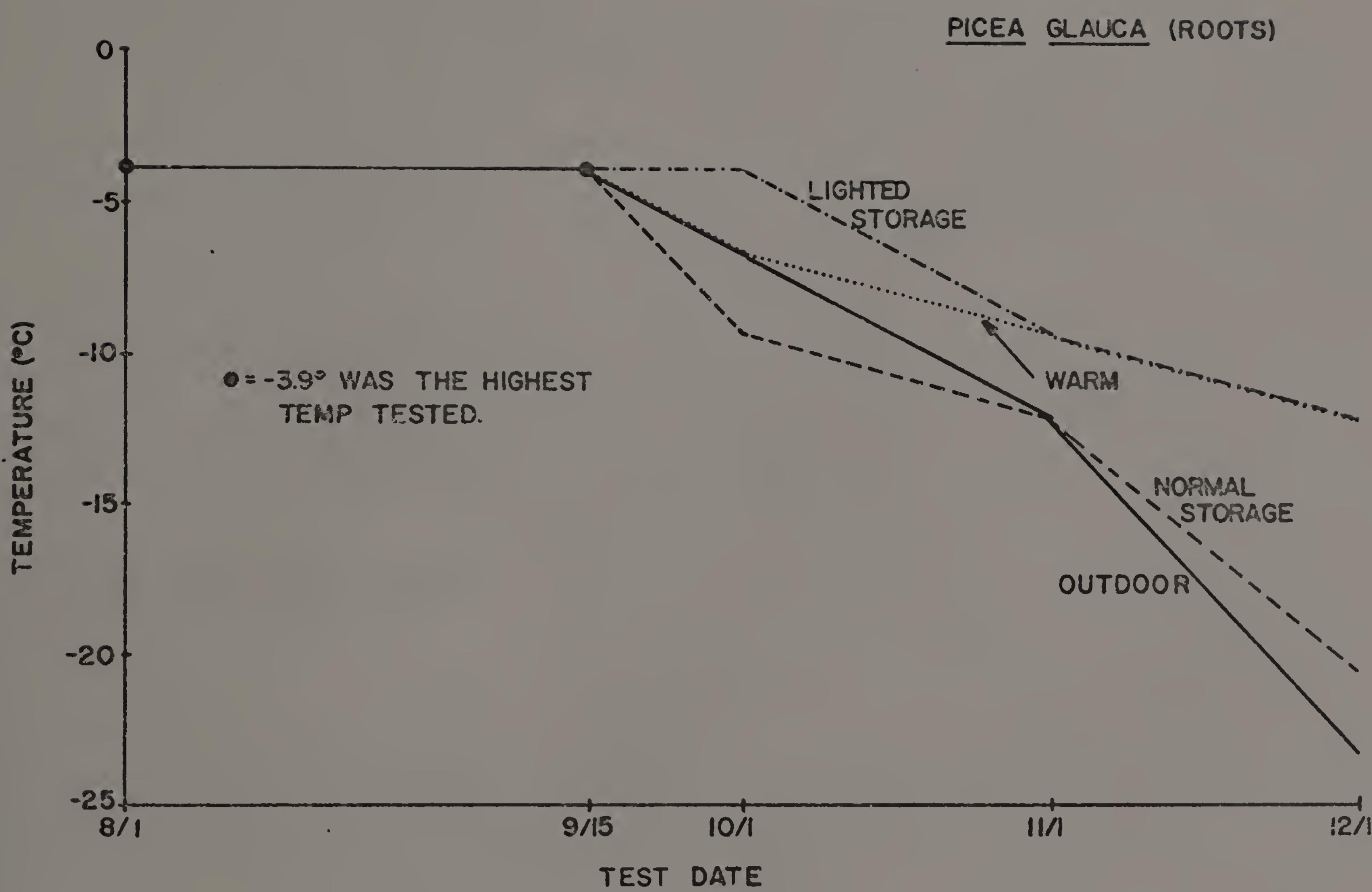

Fig. 4 Cold acclimation of Picea roots in the storage regimes described in Table 1. Lowest temp at which 50% or more secondary mature roots survived.

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SECTION II

THE EFFECT OF SUB-ZERO ROOT MEDIUM TEMPERATURE ON ROOT COLD ACCLIMATION

THE EFFECT OF SUB-ZERO ROOT MEDIUM TEMPERATURE ON ROOT COLD ACCLIMATION

Abstract. Intact roots of *Potentilla fruticosa* L. cv. Katharine Dykes and *Picea glauca* Voss were tested in Jan. to determine the importance on sub-zero medium temp on cold acclimation. Results indicated that sub-zero medium temp did not increase acclimation rates, and suggest that there is no stage-2 (freezing) cold acclimation in roots of *Potentilla*.

Cold acclimation of shoots of plants apparently follows a 2-stage system. Stage-1 seems to be influenced by decreasing daylength, while stage-2 acclimation appears to be triggered by the first autumn frost (5, 6).

There are many differences in the environment of roots when compared to shoots of plants. Some of these differences include higher humidity, modified temp, lower O_2 levels, higher CO_2 levels, and little or no light penetration to the roots. Because of these and other differences in environment, it is not surprising that roots develop less cold hardiness than shoots (3, 4, 7). It is possible that the differences in environment could also cause differences in the systems of cold acclimation of the shoots and roots.

Previous work has indicated a similar system of acclimation for the roots and the stems of plants. Pellett (4) found increased root hardiness with temp in storage at -7°C over those roots held at 2°. Mityga and Lanphear (3) presented data which supported a relationship between min air temp and root cold acclimation.

The objective of this study was to determine the importance of below 0°C medium temp on cold acclimation, and to determine if there is a stage-2 cold acclimation in roots of *Picea* and *Potentilla*.

Materials and Methods

The materials and methods used in this experiment are the same as those used by Johnson and Havis (2) with the following exceptions. For Picea, only the outdoor and normal storage treatments were used. For Potentilla, the outdoor treatment, and a modified storage treatment were used. The modified storage treatment was exposed to lighted storage regimes from Dec. 1 through Dec. 15, and after Dec. 15, was exposed to normal storage regimes until Jan. 1. The test date was Jan. 1, 1976.

Replication for the hardiness test was as follows (plants tested/temp): Picea outdoor 5, Picea normal storage 4, Potentilla outdoor 5, and Potentilla modified storage 4. This modification was necessitated by the number of plants remaining after the previous experiment.

During the test period, the outdoor treatment plants were exposed to freezing on 28 days and the min medium temp was $-22.2^{\circ}C$. The storage treatment plants did not freeze and the min medium temp was 1.7°. Minimum temp of the air and medium for the acclimating conditions are shown in Fig. 1 and 2.

Results and Discussion

Figure 3 shows the cold acclimation of Potentilla roots under the 2 conditions. Roots of plants in the outdoor treatment developed cold hardiness from $-15.0^{\circ}C$ on Dec. 1 to -34.4° on Jan. 1. The roots in the modified storage acclimated from -6.7° on Dec. 1 to -26.1 on Jan. 1. The slopes of acclimation of these two treatments are equal. If root hardiness in the lighted storage remained the same (-6.7°) from Dec. 1 until lighting was discontinued on Dec. 15 (hardiness not measured), then acclimation was even more rapid in this treatment.

These results indicate that Potentilla roots acclimated as well at a min medium temp of 1.7°C as at min medium temp of -22.2°, with natural autumn day lengths.

Figure 3 shows the cold acclimation of Picea roots under the 2 conditions. Roots in the outdoor treatment developed cold hardiness from -23.3°C on Dec. 1 to lower than -31.7° on Jan. 1. The roots in the normal storage treatment acclimated from -20.6° on Dec. 1 to lower than -28.9° on Jan. 1.

These results indicate a great degree of cold acclimation in both the outdoor and normal storage treatments, although the outdoor treatment plants were exposed to much colder temp than the normal storage plants. Because neither of the treatments were tested at temp sufficiently low to kill them, no exact comparison can be made.

Stage-2 acclimation of shoots appears to be initiated by freezing of the tissues. Sub-freezing air temp would cause freezing of shoot tissue, but freezing of the growing medium is required to freeze roots.

Mityga and Lanphear (3) indicated a close relationship between min air temp and root hardiness. These results are not in agreement with our observations. While the min air temp of the outdoor treatment was -25.6°C, the acclimation rate was no greater than the storage treatment plants which had a min air temp of 1.1°. Pellett (4) has shown increases in cold hardiness from a 2° storage to a -7° storage, when the roots were stored in sawdust. This also disagrees with our results, since we had no increase in the cold acclimation rate from a min medium temp of 1.7° to -22.2°. Differences may, however, be accounted for by the differing and fluctuating temp regimes of our plants as opposed to the stable storage temp in Pellett's work.

It appears, from the results of this study, that there is no stage-2 acclimation in the roots of *Potentilla*. Freezing temp do not appear to increase acclimation rates over those plants not frozen. These results might have been expected, when one considers the more natural root environment in the field. Although the stems are frequently exposed to subzero temp in the winter, roots of those same plants in the field rarely drop below a temp of -1.1°C (1). Since the soil temp is moderated to such a great extent, there would be no evolutionary reason for roots of plants to develop a second-stage cold acclimation mechanism, which would be useful only for very cold temp.

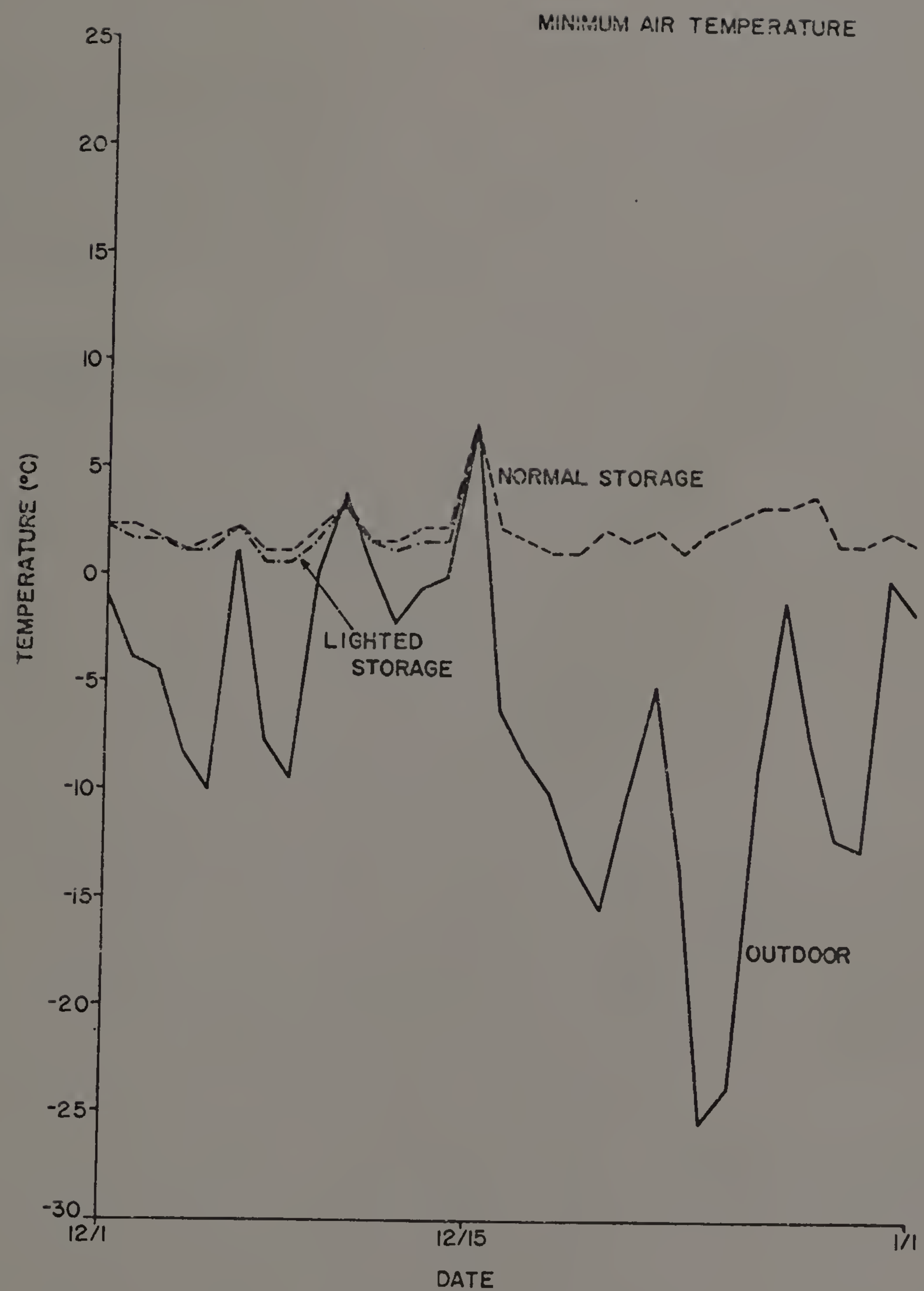

Figure 1. Minimum daily air temp exposure of Potentilla and Picea plants in storage regimes.

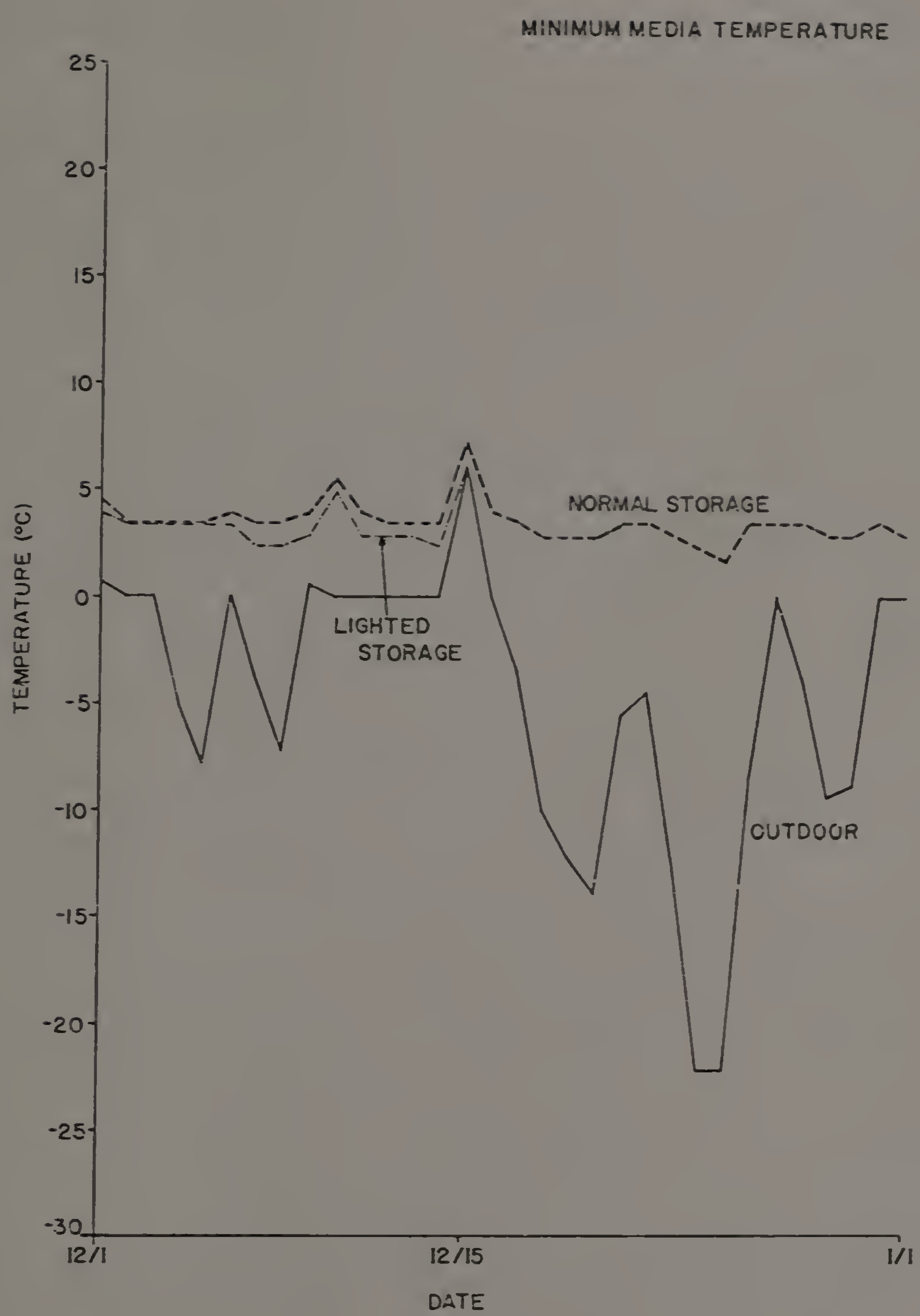

Figure 2. Minimum daily medium temp exposure of <u>Potentilla</u> and <u>Picea</u> plants in storage regimes.

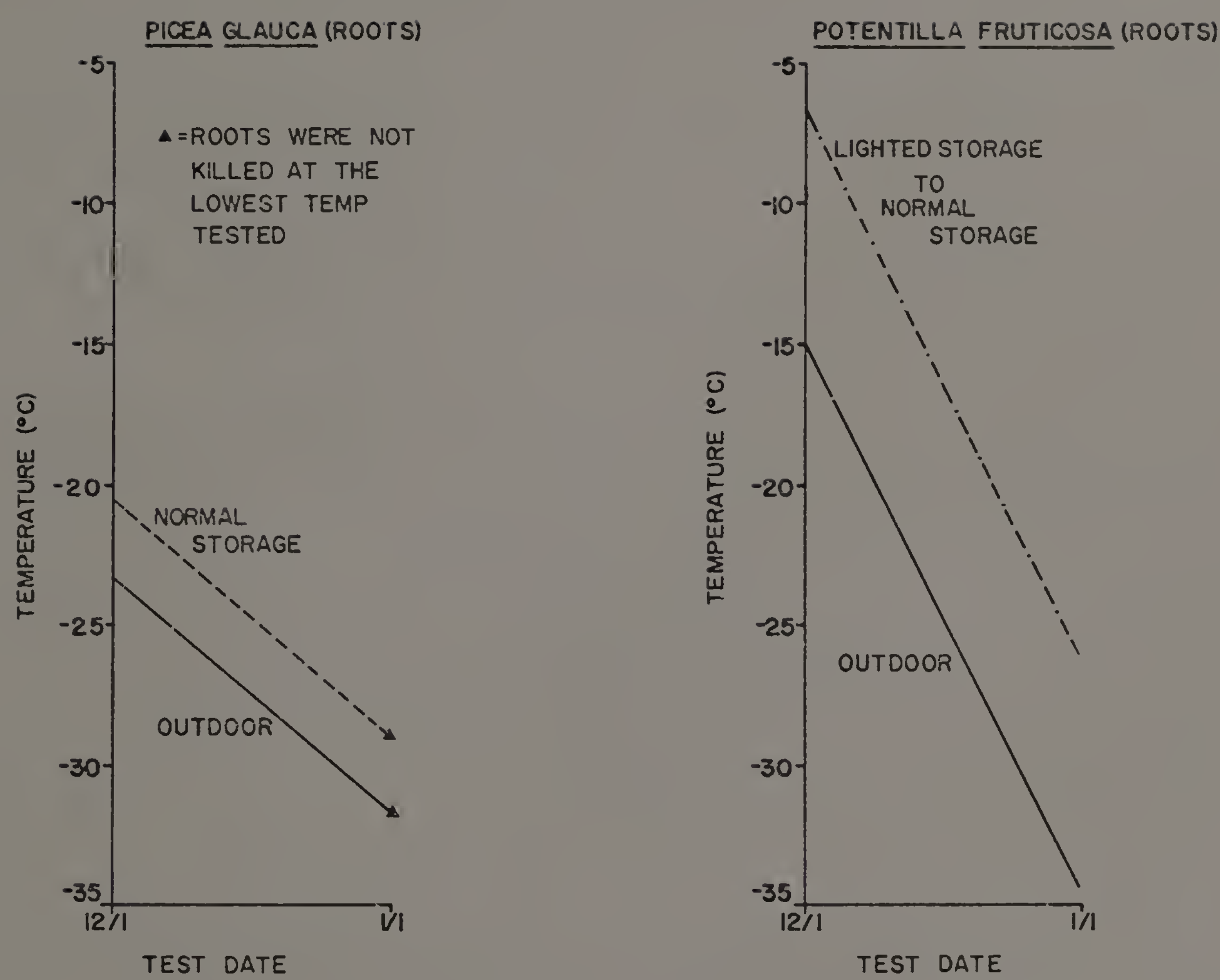

Figure 3. Cold acclimation of Picea (left) and Potentilla (right) roots in the storage regimes. Lowest temp at which 50% or more secondary mature roots survived.

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SECTION III

PHOTOPERIOD AND TEMPERATURE EFFECTS ON POTENTILLA ROOT GROWTH

PHOTOPERIOD AND TEMPERATURE EFFECTS ON POTENTILLA ROOT GROWTH

Abstract. Roots of *Potentilla fruticosa* L. cv. Katharine Dykes were counted and measured in late autumn to determine the relationship between photoperiod and temp on root growth. It appears that long days stimulate root initiation, while warm temp enhances root elongation.

Little has been written of low temp effects on root growth. The zero point of growth (ZPG) of plants has been set by Klages (4) at 4.4°C, and other workers have noted similar temp (1, 3). Barney (1) found little difference in the size of cells between 5° and 30° in loblolly pine. Stuckey (5), observing grass roots, noted cell division within 1° above the freezing point, although extensive elongation did not occur when the soil temp was less than 0°.

The purpose of this study was to determine the influence of daylength and temp on the growth of *Potentilla* roots during cold acclimation.

Materials and Methods

Potentilla plants were initially grown in translucent pots which, because of light transmission, inhibited root growth near the sides of the pots. Beginning Nov. 14, 4 plants in each treatment were removed from the white pots, observed for roots on the outside of the root ball, and then placed in green, opaque pots which allowed root growth to the sides of the pots. New or elongating roots which grew to the sides of the pots during the test period were counted and measured.

The treatments are the same as those used by Johnson and Havis (2).

Potentilla root growth is shown in Table 2. The previously proposed ZPG for plant roots of 4.4°C (3, 4) appears to be slightly higher than the ZPG for *Potentilla* roots. Minimum medium temp for the normal storage plants were between 3.3° and 3.9° during the week prior to Dec. 4 evaluation which showed little growth. During the week prior to the Nov. 21 evaluation, when elongation was much greater, min medium temp were between 3.9° and 4.4°.

The outdoor treatment plants showed no root growth during either evaluation (Table 1). The normal storage and lighted storage plants showed similar elongation rates, which could be related to the similar temp regimes received (2). The longer daylength of the lighted storage, however, appeared to initiate many more roots than in the normal storage, with the normal seasonal daylength. These results would appear to agree with previous work which indicated the presence of cell division, but restricted root elongation at temp near 0°C (5).

The warm treatment plants showed a high rate of elongation which would be expected with the warm storage temp. There were also similar numbers of roots growing when compared to the lighted storage plants. This would appear to dispute the idea that a longer daylength initiates roots. The additional roots present may be, however, roots from farther inside the root ball which have elongated to the edge of the root ball rather than initiated. Unfortunately, there was no warm-lighted treatment which, if it showed more roots growing than the warm treatment, would have supported these conclusions.

Table 1. *Potentilla* root growth.

Growth Period	Location	Avg. no. root tips growing/pot	Avg. elongation/week (cm)
Nov. 14 - Nov. 21	Normal storage	1	1.85
	Lighted storage	5	1.52
	Outdoor	0	0.0
	Warm	7	2.49
Nov. 21 - Dec. 4	Normal storage	0.25	0.25
	Lighted storage	4.5	0.38
	Outdoor	0	0.0
	Warm	5.25	1.86

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APPENDIX I

TOP GROWTH AND CHARACTER OF POTENTILLA DURING COLD ACCLIMATION

TOP GROWTH AND CHARACTER OF POTENTILLA DURING COLD ACCLIMATION

Photographs 1, 2, and 3 show typical top growth of Potentilla on Sept. 30, Nov. 5, and Dec. 1, respectively, for the 4 acclimating conditions. On Sept. 31, all treatments showed active green growth. By Nov. 5, the outdoor, normal storage, and lighted storage treatment plants showed fall color, although the lighted storage plants also had new green leaves emerging. The warm treatment plants remained green on this date, and on Dec. 1. On Dec.1, leaves on the outdoor and normal storage plants were turning brown and dropping. The lighted storage plants continued to initiate new growth, although there was fall color on the older leaves.

Since the warm treatment remained green on all dates, while fall color appeared on all of the other 3 treatments, fall color seems to be related to cool temp. Cessation of top growth appears to be a photoperiodic effect working in combination with the cool temp. The lighted storage plants, which received a long daylength, maintained some new green growth while the outdoor and normal storage plants either ceased, or nearly ceased, growth.

Fuchigami, et al. (1) presented data which indicated a hardiness promoting substance was produced by short days, and was translocated from one part of the plant top to another. It is possible that such a substance could be translocated to the roots, inducing cold hardiness. This idea is supported by the work of Mityga and Lanphear (3) in which root hardiness was reduced by girdling the plant. The reduced cold hardiness of roots from the lighted storage plants (-6.7°C) as opposed to those roots in the normal storage (-15.0°) in previous work (2), may have been due to a failure to produce such a hardiness promoting substance. Another explanation may be that there was a low ratio of hardiness promoting substance to hardiness inhibiting substance

(1). The continued terminal shoot growth in the lighted storage plants, may also have resulted in more auxin being translocated to the roots, stimulating growth and preventing cold acclimation.

Figure 1. Comparative *Potentilla* top growth on Sept. 30. Plant from storage regimes left to right: outdoor - normal storage - warm - lighted storage.

Figure 2. Comparative *Potentilla* top growth on Nov. 5. Plants from storage regimes left to right: normal storage - lighted storage - outdoor - warm.

Figure 3. Comparative *Potentilla* top growth on Dec. 1. Plants from storage regimes left to right: normal storage - lighted storage - outdoor - warm.

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APPENDIX II

ROOT CHARACTERIZATION AS DESCRIBED BY MITYGA AND LANPHEAR

Root characterization as described by Mityga and Lanphear (1).

Primary mature roots - close to the stem, dark red or brown, and is the oldest root tissue. Diameter about 2 mm.

Secondary mature roots - similar in physical appearance to the primary mature roots, but smaller in diameter and located approx halfway between the root tip and stem.

Young roots - cream to white colored roots.

Root characterization shown with branch roots stripped.

Primary mature roots - close to the stem, dark red or brown, and is the oldest root tissue. Diameter about 2 mm.

Secondary mature roots - similar in physical appearance to the primary mature roots, but smaller in diameter and located approx halfway between the root tip and stem.

Young roots - cream to white colored roots.

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APPENDIX III

SUGGESTIONS FOR FUTURE WORK

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Research on the effect of freezing temp on roots of plants has been proceeding for many years, however, there have been few researchers doing the work. As a result, there has been little work published. Below, I have listed some work that I feel should be carried out as a result of questions arising from my work.

1. Further work on the effect of sub-freezing temp on root cold acclimation should be completed to support the results I have found. I feel that this experiment should be carried through Mar. 1, hopefully, to also see some deacclimitization. I would also like to see a slightly lower storage temp, with the min between 2°C and 3°.

2. I would like to see work completed which would determine the effect of the roots on the stems and the stems on the roots during cold acclimation. I believe this could be accomplished by having the roots under cool temp (2°C to 3°) and the stems under warm temp (15° to 20°) regimes. These regimes should also be reversed in another trial, and the plants tested for cold acclimation of roots and stems. Observations of root growth would also be of interest.

3. Mityga and Lanphear (3) have listed 3 root zones which achieve different levels of hardiness. I believe some work should be done to determine the cold hardiness of each of these zones, and if possible, determine the methods of acclimation.

4. To date, I have found no objective experiment to determine the cold hardiness of roots. I have attempted to use the triphenyl tetrazolium chloride (TTC) method (4), the electrical conductance method (1), and exotherm determination of the death point (2), but none of the methods has sufficed. Wiest, et al. (5, 6)

recently used a modification of the ninhydrin method of evaluating injury. I feel that this method merits evaluation for possible later use. However, modification would be necessary so that plants could be frozen intact which, I believe, may yield more reliable results.

Literature Cited

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